Immunohistochemistry of a Gross Cystic Disease Fluid Protein (GCDFP-15) of the Breast

A Marker of Apocrine Epithelium and Breast Carcinomas With Apocrine Features

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Gross cystic disease fluid is a pathologic secretion from breast composed of several glycoproteins, including a unique 15,000-dalton monomer protein, GCDFP-15. By the immunoperoxidase technique, GCDFP-15 was localized in the apocrine metaplastic epithelium lining breast cysts and in apocrine glands in the axilla, vulva, eyelid, and ear canal. In normal breast tissue, a few individual epithelial cells within lobules and small ducts were focally positive for GCDFP-15. Fourteen of 30 breast carcinomas stained positively for GCDFP-15. Of 16 carcinomas with apocrine features, 12 stained positively. Benign and malignant lesions from other tissues, including lung, colon, ovary, endometrium, stomach, prostate, liver, esophagus, and kidney, revealed no immunoreactivity. The only cells of "non-apocrine" tissues that contained GCDFP-15 were serous cells of the submandibular salivary gland, submucosal glands of the bronchi, and accessory lacrimal glands. Phylogenetically, these tissues have biologic features in common with apocrine glands. This report is the first to characterize GCDFP-15 as a specific tissue marker of apocrine epithelium. (Am J Pathol 1983, 110:105–112)

GROSS CYSTIC DISEASE (GCD) of the breast is a premenopausal disorder in which gross cysts are the predominant pathologic lesion. Gross cysts appear to be formed from excessive apocrine cystic secretions.[1] The fluid found within these cysts is a unique secretion that only recently has been analyzed biochemically.[2-7] Four major component proteins (gross cystic disease fluid proteins, or GCDFP) have consistently been identified in the cyst fluid: 1) GCDFP-70 (70,000 daltons), immunologically human albumin; 2) GCDFP-44, zinc alpha-2-glycoprotein; 3) GCDFP-24, which binds progesterone but not cortisol and accounts for over half of the protein content of GCD fluid; and 4) GCDFP-15, which has no normal plasma counterpart but, by immunodiffusion analysis, forms a line of identity with a normal component of saliva and milk.[5]

Cytosol analysis of normal tissue specimens from all major organs has demonstrated GCDFP-15 protein in apocrine epithelia. Detectable levels of GCDFP-15 are also present in the sublingual and submaxillary salivary glands.[8] Cytosols from breast carcinoma specimens also contain GCDFP-15 in a wide range of concentrations.[8] The content is highest in more differentiated carcinomas and in those of intraductal cribiform and comedo types. Clinically, plasma levels of GCDFP-15 are useful in detecting and monitoring disease activity for approximately 40% of patients with metastatic breast carcinoma.[4]

The object of this study was to determine the specific tissue localization of GCDFP-15 in paraffin sections of normal tissues, nonneoplastic breast tissue with apocrine metaplasia, breast carcinomas with apocrine features, and a variety of other neoplastic tissue, with the use of an immunoperoxidase tech-

nique. This report is the first to characterize GCDFP-15 as a specific tissue marker of apocrine cells and to define its immunohistochemical staining profile.

Materials and Methods

Paraffin blocks of normal tissues and of benign and malignant lesions of the breast and other organ systems were selected from the surgical pathology files (1975–1981) of Brigham and Women's Hospital. The specimens were initially fixed with either 10% neutral buffered formalin or Bouin's solution.

Identification of GCDFP-15 utilized a specific rabbit antiserum[5] and the peroxidase–antiperoxidase immune complex method of Sternberger.[9] Staining was performed at room temperature with reagents equilibrated to room temperature. Tissue sections were first deparaffinized, then rehydrated in xylene and graded alcohols. Endogenous peroxidase activity was blocked by a 30-minute incubation in 0.3% hydrogen peroxide,[10] followed by a 2-minute incubation in 0.02% sodium borohydride.[11] After rinsing in phosphate-buffered saline (PBS) (0.15 M sodium chloride, 0.02 M sodium phosphate, pH 7.4), the tissues were incubated for 30 minutes with 10% normal swine serum (NSS) (Gibco, Grand Island, NY) in PBS. The slides were then drained of excess serum and wiped. The tissues were subsequently incubated for 2 hours with a 1:5000 dilution of the rabbit antiserum to GCDFP-15 diluted in 10% NSS. As a control, serial sections were incubated with a 1:1000 dilution of normal rabbit serum (NRS) diluted in 10% NSS. After rinsing with three changes of PBS for 10 minutes, a 1:40 dilution of swine anti-rabbit bridge antibody (Dako Corp, Santa Barbara, Calif) in 10% NSS was applied for 30 minutes. After rinsing, rabbit peroxidase–antiperoxidase (Dako Corporation) diluted 1:50 in 10% NSS was applied for 30 minutes. The sections were rinsed for 10 minutes in PBS; then a solution of 3,3'-diaminobenzidine tetrahydrochloride (DAB) (Sigma Chemical Company, St. Louis, Mo; 6 mg DAB in 10 ml PBS with 75 μl hydrogen peroxide) was applied for a 3-minute reaction time. The sections were then washed in water and counterstained with hematoxylin, dehydrated in graded alcohols and xylene, and mounted with Permount.

A section of axillary skin (known positive) was included as a positive control in each experiment. Primary antisera absorbed against GCDFP-15 was also substituted for the immune serum on serial sections, as a negatively staining control for specificity of the antibody staining reaction. The antiserum was absorbed by the addition of 200 μg of purified GCDFP-15 to 5 ml antiserum diluted 1:5000 and incubated for at least 2 hours at room temperature.

Results

Immunoperoxidase studies of normal tissues stained for GCDFP-15 showed GCDFP-15 localization in the apocrine epithelium of several tissues: axilla, perineum, modified sweat glands (of Moll) of the eyelid, and the ceruminous glands of the external auditory canal (Table 1). Essentially all the apocrine cells in these tissues showed both discrete supranuclear and diffuse cytoplasmic staining for GCDFP-15 (Figures 1 and 2).

In benign breast tissues (2 normal, 1 fibroadenoma, 4 gross cystic disease, 1 adenoma, 2 gynecomastia), GCDFP-15 was predominantly localized within the metaplastic apocrine epithelium lining breast cysts and in the fluid contained within these cysts (Figure 3). All cysts with apocrine epithelium stained for GCDFP-15, from the smallest microcysts to gross cysts. Staining was most intense in discrete supranuclear areas of the cells. In the large cysts where the apocrine epithelium was flattened, GCDFP-15 staining was still retained.

In normal breast tissue, GCDFP-15 staining was observed only in a few individual epithelial cells within lobules and small ducts. Positive staining for GCDFP-15, however, did occur in a fibroadenoma that had areas of apocrine metaplasia.

Fourteen of 30 breast carcinomas stained positively for GCDFP-15 (Table 2). Sixteen of the 30 carcinomas had apocrine features in hematoxylin-and-eosin–stained sections, by the following morphologic criteria: abundant acidophilic cytoplasm, cytoplasmic "snouts," and large cell size in the routine sections.[8] Twelve of these 16 carcinomas (75%) stained positively for GCDFP-15 and exhibited predominantly a diffuse cytoplasmic staining pattern (Figures 4 and 5), with an increased staining intensity adjacent

Table 1 — Localization of GCDFP-15 in Normal Non-Mammary Tissues

Tissue	Immunoreactive Cells
Axillary skin (4)*	Apocrine sweat glands
Vulva, perineum (3)	Apocrine glands
External auditory canal (1)	Apocrine glands
Eyelid (1)	Apocrine glands
	Serous cells of lacrimal gland
Salivary gland	
Submandibular (3)	Serous cells
Parotid (7)	Equivocal (3 cases)
Lung (10)	Serous cells of bronchial glands

* Number of cases evaluated in parenthesis.

to the nucleus in some neoplastic cells. Four of these 12 cases showed GCDFP-15 immunoreactivity in the majority of malignant cells. In the remaining 8 cases, there was positivity in 10–25% of the neoplastic cells.

Of the 12 cases of breast carcinoma classified as intraductal, or infiltrating with an intraductal component, all had apocrine features, and 10 stained positively for GCDFP-15. Of 12 breast carcinomas classified as infiltrating carcinomas, 4 had apocrine features, and 2 of these stained positively for GCDFP-15. However, 2 other cases of infiltrating breast carcinoma, which by histologic criteria did not have apocrine features, also stained positively for GCDFP-15. None of the cases of infiltrating lobular carcinoma or mucoid carcinoma exhibited apocrine features or stained positively for GCDFP-15.

Normal tissue and malignant tumors from bladder, colon, endometrium, esophagus, kidney, liver, lung, ovary, pancreas, prostate, skin, stomach, thyroid, and tongue did not show any immunoreactivity for GCDFP-15, with the exception of apocrine sweat
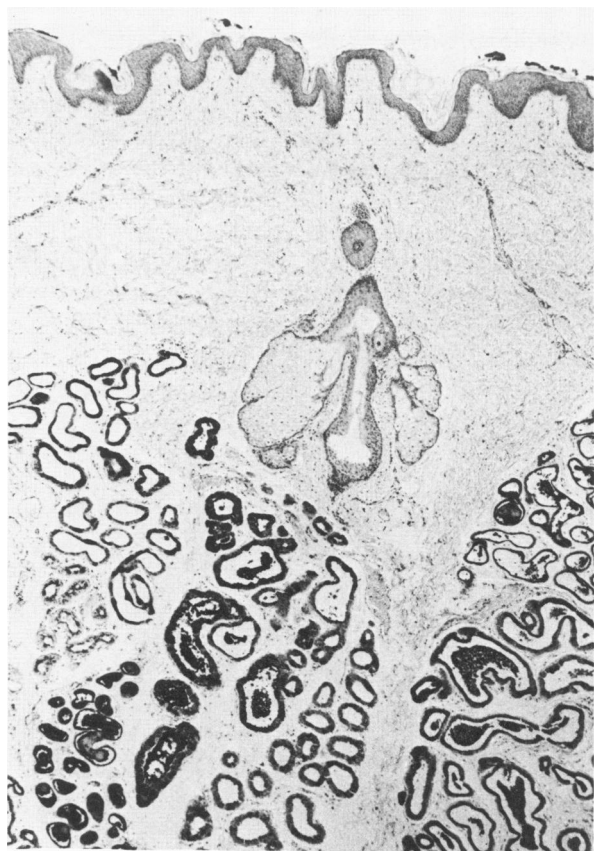

Figure 2—Skin, axilla. Higher magnification of apocrine glands demonstrates discrete supranuclear localization (*arrow*) of GCDFP-15 in many cells and focal diffuse cytoplasmic staining. (Hematoxylin counterstain, × 680)

glands in the skin and the bronchial submucosal glands (Tables 3 and 4).

The only cells of "nonapocrine" tissues that contained GCDFP-15 were serous cells of the submandibular salivary gland, submucosal glands of the bronchi, and accessory lacrimal glands (Table 1; Figure 6). Sections of parotid glands, however, showed focal weak staining in the serous glands of 3 of 7 specimens.

Discussion

Our immunoperoxidase study demonstrates tissue localization of GCDFP-15 in normal apocrine cells of the body, benign breast epithelium that has undergone apocrine metaplasia, and breast carcinoma cells with apocrine features. Thus, morphologic features shared by a specific epithelium appear to be correlated with a biochemical product, GCDFP-15. This protein serves as an excellent tissue marker for apocrine epithelium.

The unique morphology of apocrine epithelium, with its granular acidophilic cytoplasm, has been studied by anatomists for over a century. In 1876 Krause[12] noted that the "sweat glands" in skin from the axilla, eyelid, and perianal area were larger than those in other regions of the body. Extending these
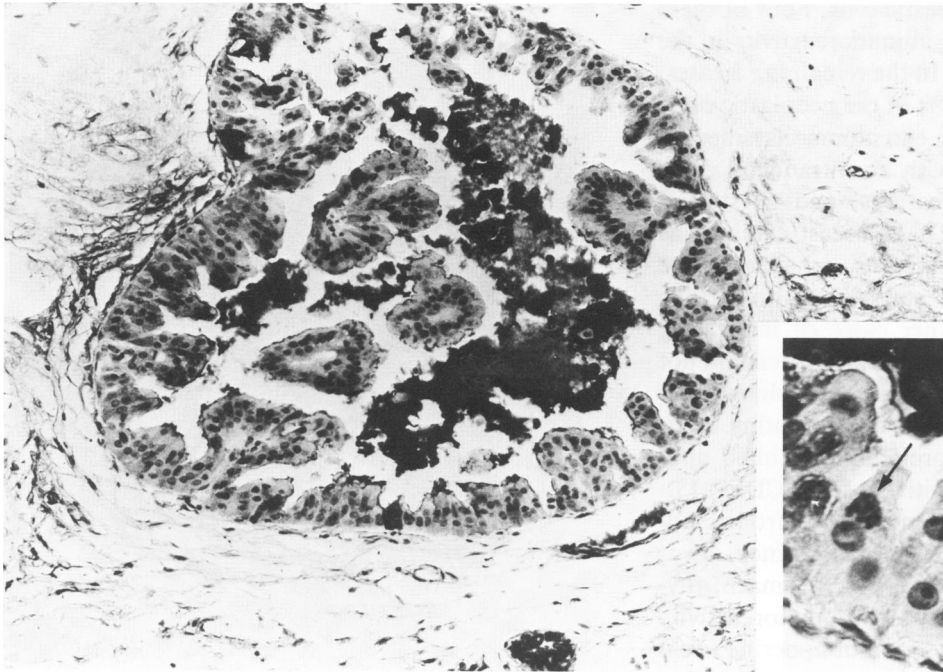

Figure 1—Skin, axilla. Immunoreactivity for GCDFP-15 is limited to apocrine glands and their lumenal contents (*black*). Other skin appendages, epidermis, and dermal collagen are negative. (Hematoxylin counterstain, × 40) (With a photographic reduction of 10%)

Figure 3— Breast, gross cystic disease. Immunoperoxidase staining of apocrine microcyst reveals strong positive staining of cyst fluid within the lumen for GCDFP-15. **Inset**—Localized supranuclear immunoreactivity (arrow) in epithelial cells lining the cyst. (Hematoxylin counterstain, x 140; **inset**, x 750)

observations, Schiefferdecker classified the cutaneous glands into holocrine (sebaceous) and merocrine.[13] The latter he divided into "apocrine" and eccrine glands. Schiefferdecker described a mechanism of secretion of apocrine cells by decapitation or sheering off of the apical projections. This mechanism has recently been confirmed by ultrastructural study.[14] Since the apocrine glands were a special type of gland under the influence of sex hormones, they have been considered as accessory sex glands or odoriforous glands.[15,16]

Apocrine glands are found in the axilla, perineum, perianal region, the ceruminous glands, tarsal glands of the eyelid (Moll's glands), and, lastly, the mammary glands.[12,15,16] Benda[17] postulated that the breast developed from a modified sweat gland. Krompecher[18] recognized that the apocrine epithelium associated with breast cysts resembled the epithelium of the apocrine glands. A variety of hypotheses have been offered to explain the presence of apocrine epithelium in the breast: 1) Apocrine epithelium is a metaplasia or incomplete differentiation of normal breast epithelium into a less developed type.[19] 2) Apocrine epithelium ("sweat glands") is normally present in the breast.[20] 3) Apocrine epithelium is the terminal phase of breast epithelial degeneration, and the cells only simulate true apocrine epithelium and are not functionally related.[21]

Studies of wholemount sections of breast have not demonstrated apocrine epithelium as a normal component of the breast duct system.[22] Histochemical and ultrastructural studies,[23-26] however, have shown characteristics of apocrine glands of the axilla to be similar to those of apocrine epithelium of the breast, supporting the hypothesis that it is a metaplastic phenomenon. Higginson and McDonald[27] compared the apocrine epithelium of the axilla with that of nonneoplastic breast tissue and apocrine carcinoma of the breast. They concluded in their study that "there is little doubt in our minds that the pale epithelium

Table 2 — Breast Carcinomas Stained for GCDFP-15

Type of carcinoma	Number of cases	Cases with apocrine features	Number stained positively
Infiltrating	12	4	4*
Infiltrating and intraductal	4	4	3
Intraductal	8	8	7
Infiltration lobular (small-cell)	4	0	0
Mucoid	1	0	0
Cystosarcoma phyllodes	1	0	0
Total	30	16	14

* Two cases with apocrine features.

Figure 4 — Breast, adenocarcinoma. Most tumor cells of this intraductal carcinoma with papillary and apocrine features exhibit cytoplasmic staining for GCDFP-15. (Hematoxylin counterstain, × 170)
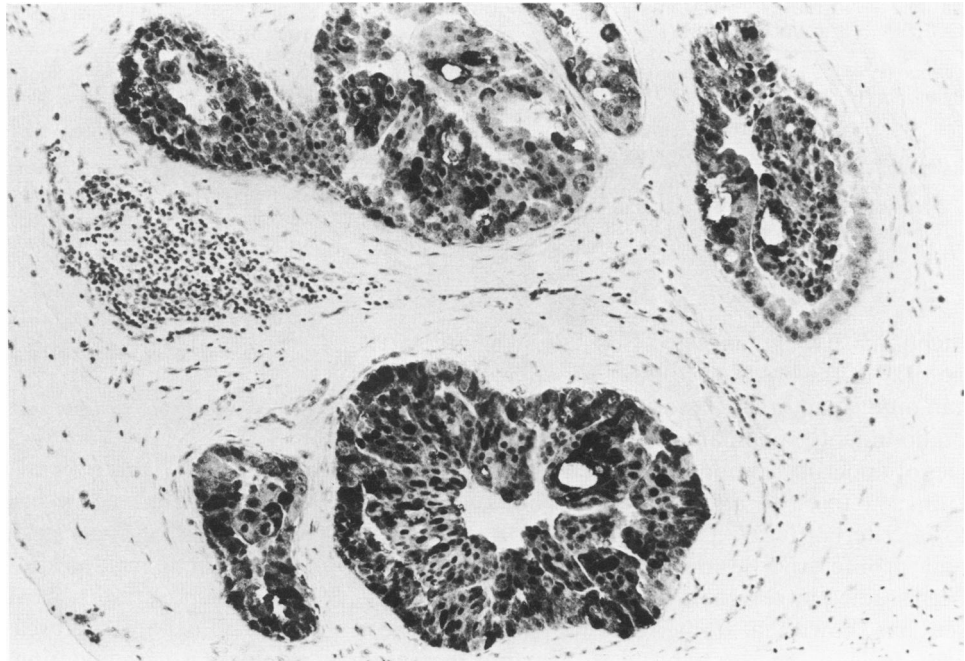
found in breasts is actually apocrine glandular tissue identical with axillary apocrine scent glands . . . Truly conclusive proof would rest on a cellular biochemical study, with identification of secretions from the tissue." The presence of a unique, secretory protein, GCDFP-15, in normal apocrine epithelium and in apocrine epithelium of the breast is strong evidence for the latter representing a metaplastic process and further implies a functional relationship of these cells.

Krompecher was the first to speculate that some breast carcinomas were derived from apocrine epi-

Figure 5 — Breast, infiltrating ductal carcinoma. Immunoperoxidase staining for GCDFP-15 reveals cytoplasmic localization in tumor cells. (Hematoxylin counterstain, × 400)
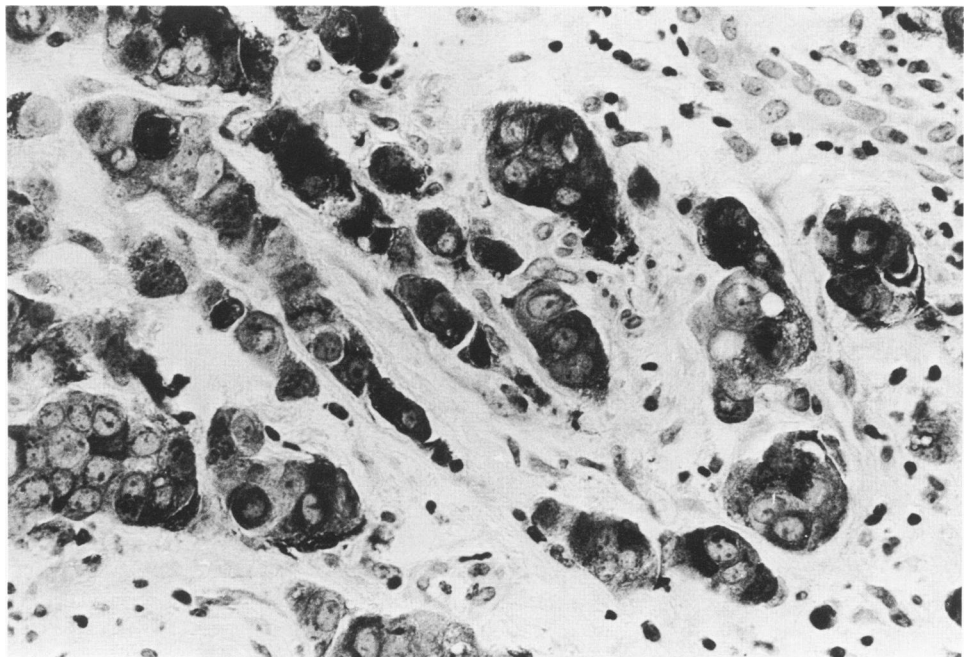
Table 3 — Normal Non-Mammary Tissues Without
GCDFP-15 Immunoreactivity

Tongue (1)*	Pancreas (1)	Endometrium (2)
Epiglottis (1)	Colon (7)	Ovary (2)
Stomach (5)	Kidney (7)	Prostate (2)
Liver (2)	Bladder (1)	Thyroid (2)
Esophagus (1)		Lung† (10)

* Number of specimens evaluated in parenthesis.
† Immunoreactivity only in serous cells of bronchial glands.

Table 4 — Non-Mammary Tumors Without GCDFP-15
Immunoreactivity

Adenocarcinoma	Squamous cell carcinoma
Esophagus (1)*	Lung (4)
Stomach (4)	Tongue (1)
Colon (5)	Basal cell carcinoma, skin (1)
Pancreas (2)	Transitional cell carcinoma, bladder (2)
Lung (3)	Cloacogenic carcinoma, rectum (2)
Kidney (5)	Hepatoma, liver (1)
Prostate (2)	Carcinoid, lung (2)
Endometrium (2)	Brenner tumor, ovary (1)
Ovary (2)	
Thyroid (1)	

* Numbers of cases evaluated in parenthesis.

thelium.[28] Ewing suggested that "sweat gland carci-
nomas" of the breast accounted for 25% of all breast
carcinomas and that these apocrine carcinomas were
predominantly papillary.[20] Other reported frequen-
cies of apocrine carcinomas of the breast have ranged
from 1% to 60%, reflecting the variability of histo-
logic criteria.[8,27,29–32] The relationship between cystic
and proliferative lesions to breast carcinoma has
been studied by several investigators.[8,27,33–37] Haagen-
sen[8] has shown that women in whom gross cystic dis-
ease develops have a twofold to fourfold increased
risk of breast carcinoma. He further noted a tenfold
increase in the risk of carcinoma in patients with
gross cystic disease with biopsy-proven microscopic
apocrine metaplasia, as compared with those lacking
this feature. The carcinomas that developed in the
group with apocrine metaplasia were of apocrine

type in 68%, and 65% of the carcinomas were intra-
ductal. These studies support a hypothesis for the
histogenesis of apocrine forms of breast carcinomas
from benign metaplastic apocrine epithelium. Our
study demonstrates localization of GCDFP-15 in
apocrine metaplastic breast epithelium and in breast
carcinomas with apocrine features but not in normal
breast epithelium. These results provide biochemical
evidence in favor of this histogenetic sequence.

The only cells of "nonapocrine" tissues containing
GCDFP-15 were the serous cells of the submandibu-
lar gland, the lacrimal gland, and the bronchial mu-
cosal glands. These tissues phylogenetically have fea-
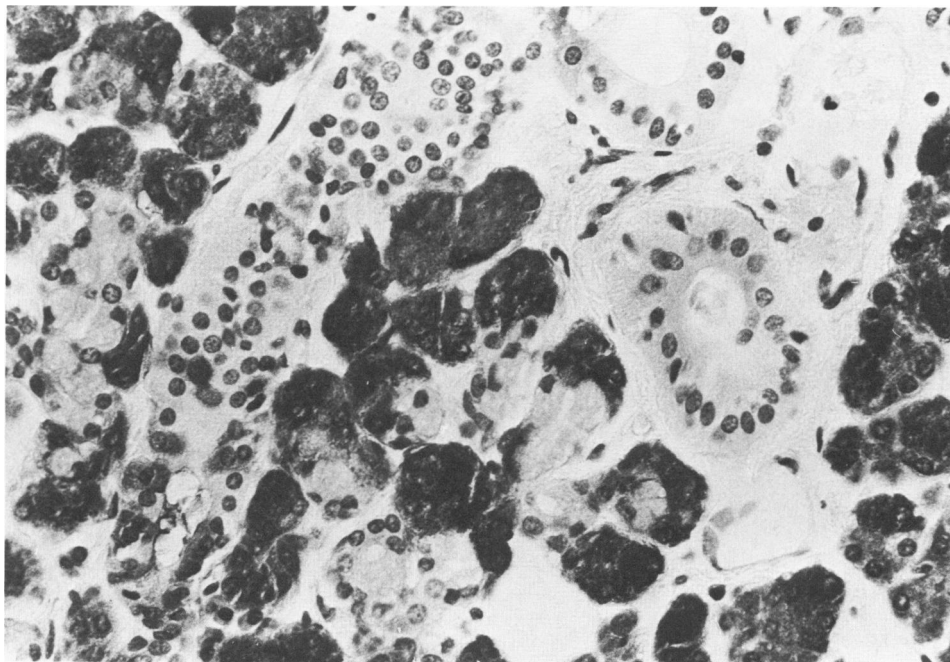
Figure 6 — Submandibular
gland. GCDFP-15 immuno-
reactivity is distinctly loca-
lized to serous cells. Mucus
cells and ductal epithelium
are clearly negative. (Hema-
toxylin counterstain, × 340)

tures in common with apocrine glands. Weak staining was also present in serous cells of some parotid glands. Parotid glands, however, may have a different embryologic origin than submandibular salivary glands.[38] It is of interest that in some species of animals the salivary glands function in the production of pheromones in a fashion similar to that of the apocrine glands.[39,40] The salivary and lacrimal gland secretions are used by some species for marking territories and for inducing sexual stimulation. Androgens have been shown to be trophic to salivary glands also in a fashion similar to that of the apocrine glands.[41,42]

Our immunohistochemical study suggests that GCDFP-15 production is a common embryologic and phylogenic property of apocrine epithelium and that GCDFP-15 is a more sensitive indicator of apocrine epithelium than morphology alone. Immunoperoxidase staining for GCDFP-15 detects this biochemical characteristic of apocrine cells even when classic morphologic features are not apparent. The discrete paranuclear localization of GCDFP-15 probably corresponds to the Golgi complex region, which is well developed in breast apocrine metaplastic cells.[23]

Our immunoperoxidase study for cellular localization of GCDFP-15 has identified a subgroup of breast carcinomas with apocrine features. The significance of this finding must be further assessed by correlation with other parameters of biologic activity such as estrogen and progesterone receptors, pattern of metastatic spread, and clinical response to therapy. This protein may represent a useful marker to suggest a possible breast origin for metastatic poorly differentiated neoplasms with unknown primary sites. Tissue localization of GCDFP-15 may also assist in defining or corroborating a possible apocrine derivation for skin tumors and in identifying subgroups of salivary gland neoplasms, particularly of submandibular origin.

References

1. Azzopardi JG: Problems in Breast Pathology. Philadelphia, W.B. Saunders, 1979
2. Bradlow ML, Schwartz MK, Fleisher M, Nisselbaum JS, Boyar R, O'Connor J, Fukushima DK: Accumulation of hormones in breast cyst fluid. J Clin Endocrinol Metab 1979, 49:778–782
3. Fleisher M, Robbins G, Breed CN, Fracchia AA, Urban JA, Schwartz MK: Biochemistry of breast cyst fluid. Memorial Sloan-Kettering Cancer Center Clinical Bulletin 1974, 4:94–97
4. Haagensen DE Jr, Mazoujian G, Holder WD Jr, Kister SJ, Wells SA Jr: Evaluation of a breast cyst fluid

5. Haagensen DE Jr, Mazoujian G, Dilley WG, Pederson CE, Kister SJ, Wells SA Jr: Breast gross cystic disease fluid analysis: I. Isolation and radioimmunoassay for a major component protein. J Natl Cancer Inst 1979, 62:239–247
6. Pearlman WH, Gueriguian JD, Sawyer ME: A specific progesterone-binding component of human breast cyst fluid. J Biol Chem 1973, 248:5736–5741
7. Pearlman WH, Peng LH, Mazoujian G, Haagensen DE Jr, Wells SA Jr, Kister SJ: A specific progesterone binding component of human breast cyst fluid: Its isolation and characterization. J Endocrinol 1977, 75:19P–20P
8. Haagensen CD, Bodian C, Haagensen DE Jr: Breast Carcinoma: Risk and Detection. Philadelphia, W.B. Saunders, 1981
9. Sternberger LA, Hardy PH, Cuculis JJ, Meyer HG: The unlabeled antibody enzyme method of immunohistochemistry. Preparation and properties of soluble antigen-antibody complex (horseradish peroxidase-antihorseradish peroxidase) and its use in identification of spirochetes. J Histochem Cytochem 1970, 18: 315–333
10. Burns J: Background staining and sensitivity of the unlabeled antibody enzyme (PAP) method. Comparison with the peroxidase labeled antibody sandwich method using formalin fixed paraffin embedded material. Histochemistry 1975, 43:291–294
11. Heyderman E, Neville AM: A shorter immunoperoxidase technique for the demonstration of carcinoembryonic antigen and other cell products. J Clin Pathol 1977, 30:138–140
12. Krause C: Handbuch der Menschlichen Anatomie, Erster Band. Allgemeine und microscopische Anatomie, Hannover 1876
13. Schiefferdecker P: Die Hautdrusen des Menschen und der Saugetiere, ihre Bedeutung sowie die Muscularis sexualis. Zoologica, 1922, 72:1–52
14. Hashimoto K, Gross B, Lever WF: Electron microscopic study of apocrine secretion. J Invest Dermatol 1966, 46:378–390
15. Shelley WB, Hurley HJ: The physiology of human axillary apocrine sweat gland. J Invest Dermatol 1953, 20:284–297
16. Way SC, Memmesheimer A: The sudoriparous glands: II. The apocrine glands. Arch Dermatol Syphilol 1938, 38:373–382
17. Benda C: Das Verhaltnis der Michdruse zu den Geschwulsten. Dermatol Z 1894, 1:94–110
18. Krompecher E: Weitere Beitrage uber das Polycystoma nammae und dessen Beziehungen zu den Geschwulsten. Virchows Arch (Pathol Anat) 1924, 250:495–516
19. Krompecher E: Uber Schweiszdrusen der Brustdruse und deren Krebse. Verh Dtsch Ges Pathol 1913, 16: 365–384
20. Ewing J: Neoplastic Diseases. 3rd edition. Philadelphia, WB Saunders, 1928
21. Dawson EK: Sweat gland carcinoma of the breast. A morpho-histological study. Edinburg Med J 1932, 39:409–438
22. Wellings SR, Jensen HM, Marcum RG: An atlas of subgross pathology of the human breast with special reference to possible precancerous lesions. J Natl Cancer Inst 1975, 55:231–243
23. Ahmed A: Apocrine metaplasia in cystic hyperplastic mastopathy. Histochemical and ultrastructural observations. J Pathol 1975, 115:211–214
24. Lendrum AC: On the "pink" epithelium of the cystic

protein detectable in the plasma of breast carcinoma patients. Ann Surg 1977, 185:279–285

breast and the staining of its granules. J Pathol 1945, 57:267-270

25. Mossler JA, Barton TK, Brinkhous AD, McCarty KJ, Moylan JA, McCarty KS Jr: Apocrine differentiation in human mammary carcinoma. Cancer 1980, 46:2463-2471

26. Tremblay G: Histochemical studies of oxidative enzymes in apocrine like cells of the breast and in axillary apocrine glands. J Invest Dermatol 1968, 50:238-243

27 Higginson JF, McDonald JR: Apocrine tissue, chronic cystic mastitis and sweat gland carcinoma of the breast. Surg Gynecol Obstet 1949, 88:1-10

28. Krompecher E: Zur Histogenese und Morphologie der Cystenmamma (maladie kystique reclus, cystadenoma Schimmelbuscin, mastitis chronica cystica Konig) des intrakanalikularen Kystadenomas und der Kystadeno-karzinome der Brustdruse (Hidrokystoma, kystadenoma, Hidrokystadenocarzinoma mammae). Beitr Pathol Anat 1916, 62:403

29. Foote FW Jr, Stewart FW: A histologic classification of carcinoma of the breast. Surgery 1946, 19:74-99

30. Frable WJ, Kay S: Carcinoma of the breast. Histologic and clinical features of apocrine tumors. Cancer 1967, 21:756-763

31. Lee BJ, Pack GT, Scharnagel I: Sweat gland cancer of the breast. Surg Gynecol Obstet 1933, 56:975-996

32. Wald M, Kakulas BA: Apocrine gland carcinoma (sweat gland carcinoma) of the breast. Aust NZ J Surg 1964, 33:200-204

33. Campbell OJ: Relationship between cystic disease of the breast and carcinoma. Arch Surg 1934, 28:1001-1056

34. Davis HH, Simons M, Davis JB: Cystic disease of the breast, relationship to carcinoma. Cancer 1964, 17:957-978

35. Foote FW, Stewart FW: Comparative studies of cancerous versus non cancerous breasts: I. Basic morphologic characteristics. Ann Surg 1945, 121:6-53

36. Monson RR, Yen S, MacMahon R, Warner S: Chronic mastitis and carcinoma of the breast. Lancet 1976, 2:224-226

37. Page DL, Swaag RV, Rogers LW, Williams LT, Walker WS, Hartman WH: Relation between component parts of fibrocystic disease complex and breast cancer. J Natl Cancer Inst 1978, 61:1055-1063

38. Batsakis JG: Tumors of the head and neck, chapter 1. Baltimore, Williams and Wilkins, 1979

39. Gower DB: 16-unsaturated C_{19} steroids. A review of their chemistry, biochemistry and possible physiological role. J Steroid Biochem 1972, 3:45-103

40. Labows JN, Preti G, Hoelzle E, Leyden J, Kilgman A: Steroid analysis of human apocrine secretion. Steroids 1979, 34:249-258

41. Brown-Grant K, Taylor W: Response of the submandibular gland of the castrated mouse to local and systemic administration of steroids. J Endocrinol 1963, 26:517-523

42. Wales NAM, Ebling FJ: The control of the apocrine glands of the rabbit by steroid hormones. J Endocrinol 1971, 51:763-770

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